

SN 09/112,904



PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : C12N 15/12, C07K 14/47, A61K 38/17, C07K 16/18, C12N 15/62</p>	<p>A2</p>	<p>(11) International Publication Number: <b>WO 99/58676</b> (43) International Publication Date: 18 November 1999 (18.11.99)</p>
<p>(21) International Application Number: PCT/US99/09831 (22) International Filing Date: 5 May 1999 (05.05.99) (30) Priority Data: 60/085,497 14 May 1998 (14.05.98) US (71) Applicant (for all designated States except US): IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): SPRIGGS, Melanie, K. [US/US]; 2256 12th Avenue West, Seattle, WA 98119 (US). (74) Agent: HENRY, Janis, C.; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> Without international search report and to be republished upon receipt of that report.</p>
<p>(54) Title: SEMAPHORIN POLYPEPTIDES</p> <p>(57) Abstract</p> <p>The invention is directed to semaphorin polypeptide as a purified and isolated protein, the DNA encoding the semaphorin polypeptide, host cells transfected with cDNAs encoding the polypeptide and methods for preparing the semaphorin polypeptide.</p>		

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## SEMAPHORIN POLYPEPTIDES

### FIELD OF THE INVENTION

The present invention relates to semaphorin polypeptides, nucleic acids encoding  
10 such semaphorin polypeptides, processes for producing recombinant semaphorin  
polypeptides, pharmaceutical compositions containing such polypeptides relates and  
processes for treating disorders associated with semaphorin activity.

### BACKGROUND OF THE INVENTION

15 The semaphorin gene family includes a large number of molecules that encode  
related transmembrane and secreted glycoproteins known to be neurologic regulators.  
The semaphorins are generally well conserved in their extracellular domains which are  
typically about 500 amino acids in length. Semaphorin family proteins have been  
observed in neuronal and nonneuronal tissue and have been studied largely for their role  
20 in neuronal growth cone guidance. For example, the secreted semaphorins known as  
collapsin-1 and Drosophila semaphorin II are selectively involved in repulsive growth  
cone guidance during development. Flies having semaphorin II genes that are mutated so  
that their function is reduced exhibit abnormal behavior characteristics.

Another semaphorin gene has been identified in several strains of poxvirus. This  
25 semaphorin is found in vaccinia virus (Copenhagen strain) and Ectromelia virus, and is  
encoded in an open reading frame (ORF) known as A39R. The A39R encoded protein  
has no transmembrane domain and no potential membrane linkage and is known to be a  
secreted protein. Variola virus ORF also contains sequences that share homology with  
the vaccinia virus ORF A39R at the nucleotide level and the amino acid level. Another  
30 viral semaphorin. AHV-sema, has been found in the Alcelaphine Herpesvirus (AHV).

Genes encoding mammalian (human, rat, and mouse) semaphorins have been  
identified, based upon their similarity to insect semaphorins. Functional studies of these  
semaphorins suggest that embryonic and adult neurons require a semaphorin to establish  
workable connections. Significantly, the fast response time of growth cone cultures to  
35 appropriate semaphorins suggests that semaphorin signaling involves a receptor-mediated  
signal transduction mechanism. Semaphorin ligands that are secreted into the  
extracellular milieu signal through receptor bearing cells in a located and systemic  
fashion. In order to further investigate the nature of cellular processes regulated by such  
local and systemic signaling, it would be beneficial to identify additional semaphorin

receptors and ligands. Furthermore, because virus encoded semaphorins are produced by infected cells and are present in viruses that are lytic (poxviruses) and viruses that are not known to be neurotropic (AHV), it is unlikely that their primary function is to modify neurologic responses. It is more likely that the virus encoded semaphorins function to  
5 modify the immunologic response of the infected host and it is likely that mammalian homologues to virus encoded semaphorins function to modify the immunologic response. In view of the suggestion that viral semaphorins may function in the immune system as natural immunoregulators it would be beneficial to identify semaphorins that may be therapeutic agents for enhancing or diminishing the immune response.

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### SUMMARY OF THE INVENTION

The present invention pertains to novel semaphorins as isolated or homogeneous proteins. In particular, the present invention provides semaphorin polypeptides, that are homologous to the viral semaphorins A39R and AHVSema. Within the scope of the  
15 present invention are DNAs encoding semaphorin polypeptides, and expression vectors that include DNA encoding semaphorin polypeptides. The present invention also includes host cells that have been transfected or transformed with expression vectors that include DNA encoding a semaphorin polypeptide, and processes for producing semaphorin polypeptides by culturing such host cells under conditions conducive to  
20 expressing semaphorin polypeptides. The present invention further includes antibodies directed against semaphorin polypeptides.

Further within the scope of the present invention are processes for purifying or separating certain novel semaphorin polypeptides or cells that express novel semaphorins to which semaphorin receptor polypeptides bind. Such processes include binding at least  
25 one semaphorin receptor to a solid phase matrix and contacting a mixture containing a semaphorin polypeptide to which the semaphorin receptor binds, or a mixture of cells expressing the semaphorin with the bound semaphorin receptor, and then separating the contacting surface and the solution.

The present invention additionally provides processes for treating mammals  
30 afflicted with a disease that is ameliorated by the interaction of semaphorins and their receptors. Such processes involve activating immune cells that express receptors for the semaphorins of the present invention and include administering a therapeutically effective amount of semaphorin to a mammal afflicted with the disease. The therapeutically effective amount is sufficient to activate immune system cells that express semaphorin  
35 receptors. Such an activation results in the secretion of cytokines, the regulation of activation antigens or the migration of the cell to sites of immune activity.

### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel semaphorin polypeptides, DNA encoding semaphorin polypeptides and recombinant expression vectors that include DNA encoding semaphorin polypeptides. The present invention further provides methods for isolating semaphorin polypeptides and methods for producing recombinant semaphorin polypeptides by cultivating host cells transfected with the recombinant expression vectors under conditions appropriate for expressing semaphorin and recovering the expressed semaphorin polypeptide.

This invention additionally provides antibodies directed against semaphorin polypeptides.

Particular semaphorin embodiments of the present invention include polypeptides homologous to AHVsema. The native semaphorin polypeptide described herein was discovered using data base search and comparison techniques that resulted in the identification of at least one EST having some homology to viral semaphorins. As described in Example 1, PCR techniques were used to identify and clone the full viral semaphorin homologue. The human semaphorin of the present invention is found in placenta, testis, ovary, spleen, dendritic cells, and B cells. Semaphorin polypeptides of the present invention bind to the semaphorin receptor, designated VESPR (described in copending application S/N 08/958,598, incorporated herein by reference). Evidence suggests that the interaction between the semaphorins of the present invention and their receptors are associated with the immune suppression of mature dendritic cells. For this reason, the semaphorins of the present invention are designated DCSema.

Example 1 describes identifying a native DCSema of the present invention. The amino acid sequence of the identified native DCSema is disclosed in SEQ ID NO:2 and the DNA encoding the amino acid sequence is disclosed in SEQ ID NO:1. The amino acid sequence presented in SEQ ID NO:2 is a secreted soluble polypeptide, but may additionally exist as a membrane bound protein. The amino acid sequence of SEQ ID NO:2 has a predicted signal sequence that includes amino acids 1-44. Also encompassed within the present invention are soluble DCSema polypeptides that lack the signal sequence. An example of such a polypeptide is amino acids 45-666 of SEQ ID NO:2. The EST 151129 portion of SEQ ID NO:2 has a 28% identity to A39R and 44% identity to AHVsema, both of which bind to VESPR, a semaphorin receptor described in copending patent application S/N 08/958,598. A39R and AHVsema share 29% identity.

The terms "semaphorin polypeptide", "human semaphorin homologue", "DCSema" and homologues of AHVsema encompass polypeptides having the amino acid sequence disclosed in SEQ ID NO:2, and proteins that are encoded by nucleic acids that contain the nucleic acid sequence of SEQ ID NO:1. In addition, those polypeptides

that have a high degree of similarity or a high degree of identity with the amino acid sequence of SEQ ID NO:2, which polypeptides are biologically active and bind at least one molecule or fragments of a molecule that is a semaphorin receptor. In addition, "semaphorin polypeptide", "human semaphorin homologue", "DCSema" refers to biologically active gene products of the DNA of SEQ ID NO:1. Further encompassed by semaphorin polypeptides are soluble or truncated proteins that comprise primarily the binding portion of the protein, retain biological activity and are capable of being secreted. Specific examples of such soluble proteins are those comprising the sequence of amino acids 45-666 of SEQ ID NO:2.

The term "biologically active" as it refers to semaphorin polypeptides, means that the semaphorin polypeptide is capable of binding to at least one semaphorin receptor. Assays suitable for determining DCSema binding are described infra and can include standard flow cytometry tests and slide binding tests.

"Isolated" means a DCSema polypeptide is free of association with other proteins or polypeptides, for example, as a purification product of recombinant host cell culture or as a purified extract.

A DCSema polypeptide variant as referred to herein, means a polypeptide substantially homologous to native DCSema polypeptide, but which has an amino acid sequence different from that of the native polypeptides because of one or more deletions, insertions or substitutions. The variant amino acid sequence preferably is at least 80% identical to a native DCSema amino acid sequence, most preferably at least 90% identical. The percent identity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Variants may comprise conservatively substituted sequences, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu. or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity

characteristics, are well known. Naturally occurring variants or alleles are also encompassed by the invention. Examples of such variants are proteins that result from alternate mRNA splicing events or from proteolytic cleavage of the DCSema protein, wherein the binding property is retained. Alternate splicing of mRNA may yield a truncated but biologically active polypeptide, such as a naturally occurring soluble form of the protein, for example. Variations attributable to proteolysis include, for example, differences in the N-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the DCSema polypeptide (generally from 1-5 terminal amino acids).

Example 3 describes the construction of a novel viral Semaphorin/Fc fusion (DCSema/Fc) protein that may be utilized in studying the biological characteristics of DCSema and their receptor distribution. Other antibody Fc regions may be substituted for the human IgG1 Fc region described in Example 3. Suitable Fc regions are those that can bind with high affinity to protein A or protein G, and include the Fc region of human IgG1 or fragments of the human or murine IgG1 Fc region, e.g., fragments comprising at least the hinge region so that interchain disulfide bonds will form. The viral DCSema/Fc fusion protein offers the advantage of being easily purified. In addition, disulfide bonds form between the Fc regions of two separate fusion protein chains, creating dimers.

The soluble DCSema polypeptides of the present invention may be isolated and identified by separating intact cells that express the desired protein from the culture medium in which the cells grow, and then assaying the medium (supernatant) for the presence of the desired protein. Separation can be accomplished using standard separation techniques including, including centrifugation. The presence of the DCSema polypeptide in the medium indicates that the protein was secreted from the cells in its expected soluble form. Because the DCSema polypeptides of the present invention are secreted soluble polypeptides they possess many advantages over membrane bound proteins. Purification of the proteins from recombinant host cells is feasible, since the soluble proteins are secreted from the cells. Further, soluble proteins are generally more suitable for intravenous administration.

Truncated DCSema proteins comprising less than the entire secreted polypeptide are included in the invention, e.g. soluble fragments such as amino acids 52-543 of SEQ ID NO:2 that include the "semaphorin domain," which is part of an active binding site, and amino acids 45-644 of SEQ ID NO:2 that include the secreted protein without the signal peptide, are included in the invention. When initially expressed within a host cell, DCSema polypeptides may additionally comprise one of the heterologous signal peptides described below that is functional within the host cells employed. Alternatively, the protein may comprise the native signal peptide. In one embodiment of the invention,

DCSema polypeptides can be expressed as a fusion protein comprising (from N- to C-terminus) the yeast  $\alpha$ -factor signal peptide, a FLAG<sup>®</sup> peptide described below and in U.S. Patent No. 5,011,912, and soluble DCSema polypeptide consisting of amino acids 45 to 666 of SEQ ID NO:2. This recombinant fusion protein is expressed in and secreted from yeast cells. The FLAG<sup>®</sup> peptide facilitates purification of the protein, and subsequently may be cleaved from the DCSema using bovine mucosal enterokinase.

Truncated DCSema polypeptides may be prepared by any of a number of conventional techniques. A desired DNA sequence may be chemically synthesized using techniques known *per se*. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. Linkers containing restriction endonuclease cleavage site(s) may be employed to insert the desired DNA fragment into an expression vector, or the fragment may be digested at cleavage sites naturally present therein. The well known polymerase chain reaction procedure also may be employed to amplify a DNA sequence encoding a desired protein fragment. As a further alternative, known mutagenesis techniques may be employed to insert a stop codon at a desired point, e.g., immediately downstream of the codon for the last amino acid of the binding domain.

As stated above, the invention provides isolated or homogeneous DCSema polypeptides, both recombinant and non-recombinant. Variants and derivatives of native DCSema proteins that retain the desired biological activity (e.g., the ability to bind to DCSema receptors) may be obtained by mutations of nucleotide sequences coding for the native polypeptides. Alterations of the native amino acid sequence may be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985); Kunkel et al. (*Methods in Enzymol.* 154:367, 1987); and U.S. Patent Nos. 4,518,584 and 4,737,462 all of which are incorporated by reference.

Native DCSema polypeptides may be modified to create DCSema derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as



glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of the polypeptides may be prepared by linking the chemical moieties to functional groups on DCSema amino acid side chains or at the N-terminus or C-terminus of a DCSema polypeptide. Other derivatives of the polypeptides within the scope of this invention include covalent or aggregative conjugates of DCSema polypeptides or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugate may comprise a signal or leader polypeptide sequence (e.g. the  $\alpha$ -factor leader of *Saccharomyces*) at the N-terminus of a polypeptide of the present invention. The signal or leader peptide co-translationally or post-translationally directs transfer of the conjugate from its site of synthesis to a site inside or outside of the cell membrane or cell wall.

DCSema polypeptide fusion proteins can comprise peptides added to facilitate purification and identification of semaphorin receptors that bind semaphorins of the present invention, including VESPR (see copending application S/N08/958,598. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988.

The invention further includes DCSema polypeptides with or without associated native-pattern glycosylation. DCSema polypeptide expressed in yeast or mammalian expression systems (e.g., COS-7 cells) may be similar to or significantly different from a native DCSema polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of DCSema polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules.

Equivalent DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed for biological activity or binding are encompassed by the invention. For example, N-glycosylation sites in the DCSema extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. The native human DC Sema of the present invention comprises 4 such amino acid triplets, at amino acids 105-107, 157-159, 258-260 and 602-604 of SEQ ID NO:2. Appropriate substitutions, additions or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Known procedures for inactivating N-glycosylation sites in proteins

include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

In another example, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon renaturation. Other equivalents are prepared by modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites. The DC sema of SEQ ID NO:2 contains 7 KEX2 protease processing sites at amino acids 123-124, 135,136, 192,193, 204-205, 460-461, 474-475 and 475-476,

Nucleic acid sequences within the scope of the invention include isolated DNA and RNA sequences that hybridize to DCSema nucleotide sequences disclosed herein under conditions of moderate or high stringency, and that encode biologically active DCSema. Conditions of moderate stringency, as defined by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press, (1989), include use of a prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of about 55°C, 5 X SSC, overnight. Conditions of severe stringency include higher temperatures of hybridization and washing. The skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as the length of the nucleic acid molecule and the relative amount of A, T/U, C and G nucleotides.

Due to the known degeneracy of the genetic code wherein more than one codon can encode the same amino acid, a DNA sequence may vary from that shown in SEQ ID NO:1 and still encode a polypeptide having the amino acid sequence of SEQ ID NO:2. Such variant DNA sequences may result from silent mutations (e.g., occurring during PCR amplification), or may be the product of deliberate mutagenesis of a native sequence.

The invention provides equivalent isolated DNA sequences encoding biologically active human DCSema, selected from: (a) cDNA comprising the nucleotide sequence presented in SEQ ID NO:1; (b) DNA capable of hybridization to a DNA of (a) under moderately stringent conditions and that encodes biologically active polypeptides and (c) DNA that is degenerate as a result of the genetic code to a DNA defined in (a), or (b) and

that encodes biologically active human DCSema polypeptide. Polypeptides encoded by such DNA equivalent sequences are encompassed by the invention.

DNAs that are equivalent to the DNA sequence of SEQ ID NO:1 include those that hybridize under moderately and highly stringent conditions to the DNA sequence that encodes polypeptides comprising the sequence of SEQ ID NO:2. Examples of  
5 proteins encoded by such DNA, include, but are not limited to, polypeptide fragments and proteins that have inactivated N-glycosylation site(s), inactivated KEX2 protease processing site(s), or conservative amino acid substitution(s), as described above. DCSema polypeptides encoded by DNA derived from other species, wherein the DNA  
10 will hybridize to the cDNA of SEQ ID NO:1, are also within the present invention.

DCSema polypeptide variants possessing the ability to bind semaphorin receptors may be identified by any suitable assay. Biological activity of DCSema polypeptides of the present invention may be determined, for example, by competition for binding to the binding domain of semaphorin receptors, e.g. competitive binding assays, or for binding  
15 to a semaphorin receptor binding domain.

One type of a competitive binding assay for a DCSema polypeptide of the present invention uses a radiolabeled DCSema and intact semaphorin receptor-expressing cells. Instead of intact cells, one could substitute soluble semaphorin receptor:Fc fusion proteins bound to a solid phase through the interaction of a Protein A, Protein G or an  
20 antibody to the semaphorin receptor or Fc portions of the molecule, with the Fc region of the fusion protein. Competitive binding assays can be performed following conventional methodology. In one embodiment, a soluble semaphorin receptor can be made to compete with an immobilized receptor for binding with a soluble semaphorin ligand. For example, a radiolabeled soluble semaphorin ligand can be antagonized by soluble VESPR  
25 in an assay for binding activity against a surface-bound semaphorin receptor. Qualitative results can be obtained by competitive autoradiographic plate binding assays, or Scatchard plots may be utilized to generate quantitative results.

Alternatively, semaphorin binding proteins, such as VESPR or anti-semaphorin antibodies, can be bound to a solid phase such as a column chromatography matrix or a  
30 similar substrate suitable for identifying, separating or purifying cells that express semaphorin on their surface. Binding of a semaphorin-binding protein to a solid phase contacting surface can be accomplished by any means, for example, by constructing a VESPR:Fc fusion protein and binding such to the solid phase through the interaction of Protein A or Protein G. Various other means for fixing proteins to a solid phase are well  
35 known in the art and are suitable for use in the present invention. For example, magnetic microspheres can be coated with VESPR and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures containing semaphorin-expressing cells are

contacted with the solid phase that has VESPR polypeptides thereon. Cells having semaphorin on their surface bind to the fixed VESPR and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening or separating such semaphorin-expressing cells from solution. Methods of releasing positively selected cells  
5 from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner. In the case of semaphorin-VESPR interactions, the enzyme preferably would cleave the semaphorin, thereby freeing the resulting cell suspension from the "foreign" semaphorin receptor  
10 material. The purified cell population then may be used to repopulate mature (adult) tissues.

Alternatively, mixtures of cells suspected of containing semaphorin cells first can be incubated with a suitable biotinylated semaphorin receptor, e.g. VESPR. Incubation periods are typically at least one hour in duration to ensure sufficient binding to  
15 semaphorin. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the cell to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.*, 10D:239 (1986). Washing unbound material and releasing the bound cells is performed using conventional methods.

As described above, cells expressing DCSema polypeptides of the present invention can be separated using a semaphorin receptor, e.g. VESPR. In an alternative method, VESPR, other suitable semaphorin receptors, or an extracellular domain or a fragment thereof can be conjugated to a detectable moiety such as  $^{125}\text{I}$  to detect  
20 semaphorin-expressing cells. Radiolabeling with  $^{125}\text{I}$  can be performed by any of several standard methodologies that yield a functional  $^{125}\text{I}$ -molecule labeled to high specific activity or an iodinated or biotinylated antibody against the semaphorin receptor. Another detectable moiety such as an enzyme that can catalyze a colorimetric or  
25 fluorometric reaction, biotin or avidin may be used. Cells to be tested for DCSema polypeptide-expression can be contacted with a suitable labeled receptor, e.g. VESPR. After incubation, unbound labeled receptor is removed and binding is measured using the  
30 detectable moiety.

The binding characteristics of DCSema polypeptides may also be determined using a conjugated semaphorin receptor (for example,  $^{125}\text{I}$ -semaphorin receptor:Fc) in competition assays similar to those described above. In this case, however, intact cells  
35 expressing DCSema polypeptide of the present invention, bound to a solid substrate, are used to measure the extent to which a sample containing a putative receptor variant competes for binding with a conjugated DCSema.

Other means of assaying for DCSema polypeptides of the present invention include the use of anti-DCSema antibodies, cell lines that proliferate in response to DCSema polypeptide, or recombinant cell lines that express DCSema and proliferate in the presence of a suitable semaphorin receptor.

5       The DCSema proteins disclosed herein also may be employed to measure the biological activity of any semaphorin receptor in terms of its binding affinity for its semaphorin ligand. As one example, DCSema polypeptides of the present invention may be used in determining whether biological activity is retained after modification of a semaphorin receptor (e.g., chemical modification, truncation, mutation, etc.). The  
10       biological activity of a semaphorin receptor thus can be ascertained before it is used in a research study, or in the clinic, for example.

DCSema polypeptides disclosed herein find use as reagents in "quality assurance" studies, e.g., to monitor shelf life and stability of a receptor to which the DCSema binds under different conditions. To illustrate, DCSema polypeptides of the present invention  
15       may be employed in a binding affinity study to measure the biological activity of a test semaphorin receptor that has been stored at different temperatures, or produced in different cell types. The binding affinity of the DCSema protein for the test receptor is compared to that of a standard or control semaphorin receptor to detect any adverse impact on biological activity of the test semaphorin receptor.

20       DCSema polypeptides described herein also find use as carriers for delivering agents attached thereto to cells expressing semaphorin receptors to which the semaphorin binds. As described in copending application S/N 958,598, VESPR, to which a DCSema of the present invention binds, is expressed in lung epithelial cells, stroma, intestinal epithelial cells and lymphoma cells. DCSema polypeptides of the present invention can  
25       thus can be used to deliver diagnostic or therapeutic agents to these cells (or to other cell types found to express a suitable semaphorin receptor on cell surfaces) in *in vitro* or *in vivo* procedures.

Diagnostic and therapeutic agents that may be attached to a DCSema polypeptide of the present invention include, but are not limited to, drugs, toxins, radionuclides,  
30       chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Examples of drugs include those used in treating various forms of cancer, e.g., nitrogen mustards such as L-phenylalanine nitrogen mustard or cyclophosphamide, intercalating agents such as cis-diaminodichloroplatinum, antimetabolites such as 5-fluorouracil, vinca alkaloids  
35       such as vincristine, and antibiotics such as bleomycin, doxorubicin, daunorubicin, and derivatives thereof. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as

trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to,  $^{123}\text{I}$ ,  $^{131}\text{I}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ , and  $^{76}\text{Br}$ . Radionuclides suitable for therapeutic use include, but are not limited to,  $^{131}\text{I}$ ,  $^{211}\text{At}$ ,  $^{77}\text{Br}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$ ,  $^{109}\text{Pd}$ ,  $^{64}\text{Cu}$ , and  $^{67}\text{Cu}$ .

5        Such agents may be attached to the DCSema of the present invention by any suitable conventional procedure. Semaphorin homologues of the present invention, being proteins, include functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional  
10        group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to the receptor by using a suitable bifunctional chelating agent, for example. Conjugates comprising molecules of the  
15        present invention and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Another use of the DCSema polypeptides of the present invention is as a research tool for studying the role that the DCSema, in conjunction with semaphorin receptors to which it binds, may play in immune regulation and viral infection. The polypeptides of the present invention also may be employed in *in vitro* assays for detection of receptors to which it binds, e.g. VESPR, or the interactions thereof. Similarly, DCSema polypeptides of the present invention can be used as a research tool for studying the role that the ligand, in conjunction with its receptors, play in immune regulation.

25        Furthermore, it is known that administration of IL-12 to tumor bearing animals results in tumor regression and the establishment of a tumor-specific immune response. Thus, using a DCSema ligand to bind with VESPR in order to enhance or promote IL-12 can induce a curative immune response against aggressive micrometastasizing tumors.

VESPR, a semaphorin receptor, binds with a binding partner to downregulate expression of MHC Class II molecules and CD86, a co-stimulatory molecule, on dendritic cells, cultured with GM-CSF and IL-4 (see copending application S/N 60/085,497). By analogy, this suggests that the interaction between DCSema of the present invention and its receptors are associated with the immune suppression of mature dendritic cells. Thus, the use of DCSema ligands, including the DC Sema of the present  
30        invention, in the treatment of autoimmune disorders is expected to diminish unwanted symptoms associated with the autoimmune disorder by downregulating the antigen presenting capabilities of mature dendritic cells.  
35

DCSema polypeptides of the invention can be formulated according to known methods used to prepare pharmaceutically useful compositions. The molecules of the invention can be combined in admixture, either as the sole active material or with other known active materials, with pharmaceutically suitable diluents (e.g., Tris-HCl, acetate, phosphate), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences, 16th ed. 1980, Mack Publishing Co. In addition, such compositions can contain DCSema polypeptide complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance of DCSema. DCSema polypeptides described herein can be conjugated to antibodies against tissue-specific receptors, ligands or antigens, or coupled to ligands of tissue-specific receptors.

DCSema polypeptides of the present invention can be administered topically, parenterally, or by inhalation. The term "parenteral" includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. These compositions will typically contain an effective amount of the polypeptide, alone or in combination with an effective amount of any other active material. Such dosages and desired drug concentrations contained in the compositions may vary depending upon many factors, including the intended use, patient's body weight and age, and route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration can be performed according to art-accepted practices.

Polypeptides of the present invention may exist as oligomers, such as covalently-linked or non-covalently-linked dimers or trimers. Oligomers may be linked by disulfide bonds formed between cysteine residues on different DCSema molecules. In one embodiment of the invention, a dimer is created by fusing a DCSema to the Fc region of an antibody (e.g., IgG1) in a manner that does not interfere with binding of the DCSema to a semaphorin receptor-binding domain. The Fc polypeptide preferably is fused to the C-terminus of a DCSema. General preparation of fusion proteins comprising heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991) and Byrn et al. (*Nature* 344:677, 1990), hereby incorporated by reference. A gene fusion encoding the DCSema/Fc fusion protein is inserted into an appropriate expression vector. DCSema/Fc fusion proteins are allowed to assemble much

like antibody molecules, whereupon interchain disulfide bonds form between Fc polypeptides, yielding divalent . If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a DCSema oligomer with as many as four semaphorin regions. Alternatively, one can link two DCSemas with a peptide linker.

5        Suitable host cells for expression of DCSema polypeptides of this invention include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce the  
10       polypeptides of the present invention using RNAs derived from DNA constructs disclosed herein.

      Prokaryotes include gram negative or gram positive organisms, for example, *E. coli* or *Bacilli*. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the  
15       genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, a semaphorin receptor polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant DCSema polypeptide.

20       DCSema polypeptides may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia*, *K. lactis* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2 $\mu$  yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for  
25       transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate  
30       decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657 or in Fleer et. al., *Gene*, 107:285-195 (1991); and van den Berg et. al., *Bio/Technology*, 8:135-139 (1990). Another alternative is the  
35       glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection



and replication in *E. coli* (Amp<sup>r</sup> gene and origin of replication) into the above-described yeast vectors.

The yeast  $\alpha$ -factor leader sequence may be employed to direct secretion of the VESPR or DCSema polypeptide. The  $\alpha$ -factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. See, e.g., Kurjan et al., *Cell* 30:933, 1982; Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984; U. S. Patent 4,546,082; and EP 324,274. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978. The Hinnen et al. protocol selects for Trp<sup>+</sup> transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10  $\mu$ g/ml adenine and 20  $\mu$ g/ml uracil.

Yeast host cells transformed by vectors containing ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80  $\mu$ g/ml adenine and 80  $\mu$ g/ml uracil. Depression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems could also be employed to express recombinant polypeptides of the present invention. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell* 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV-1/EBNA-1 cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Transcriptional and translational control sequences for mammalian host cell expression vectors may be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from Polyoma virus, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are

particularly useful because both are easily obtained from a viral genome as a fragment which may also contain a viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Exemplary expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984 has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in U.S. Patent Application Serial No. 07/701,415, filed May 16, 1991, incorporated by reference herein. The vectors may be derived from retroviruses. In place of the native signal sequence, and in addition to an initiator methionine, a heterologous signal sequence may be added, such as the signal sequence for IL-7 described in United States Patent 4,965,195; the signal sequence for IL-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the IL-4 signal peptide described in EP 367,566; the type I IL-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II IL-1 receptor signal peptide described in EP 460,846.

DCSema polypeptides of the present invention, as isolated, purified or homogeneous proteins may be produced by recombinant expression systems as described above or purified from naturally occurring cells. The polypeptides can be purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

One process for producing polypeptides of the present invention comprises culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes the desired DCSema polypeptide under conditions sufficient to promote expression of the DCSema polypeptide. The DCSema polypeptide is then recovered from culture medium or cell extracts, depending upon the expression system employed. As is known to the skilled artisan, procedures for purifying a recombinant protein will vary according to such factors as the type of host cells employed and whether or not the recombinant protein is secreted into the culture medium.

For example, when expression systems that secrete the recombinant protein are employed, the culture medium first may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification

matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel having pendant methyl or other aliphatic groups) can be employed to further purify the polypeptide. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide a substantially homogeneous recombinant protein.

It is possible to utilize an affinity column comprising the receptor-binding domain of a DCSema of the present invention affinity-purify semaphorin receptor to which the DCSema binds. Such a receptor can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized.

Recombinant protein produced in bacterial culture can be isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Transformed yeast host cells are preferably employed to express DCSemas of the present invention as a secreted polypeptide in order to simplify purification. Secreted recombinant polypeptide from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

Useful fragments of the DCSema nucleic acids of the present invention include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target DCSema mRNA (sense) or DCSema DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of DCSema cDNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based

upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

5 Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target  
sequence by one of several means, including enhanced degradation of the duplexes,  
premature termination of transcription or translation, or by other means. The antisense  
oligonucleotides thus may be used to block expression of proteins of the present  
invention. Antisense or sense oligonucleotides further comprise oligonucleotides having  
modified sugar-phosphodiester backbones (or other sugar linkages, such as those  
10 described in WO91/06629) and wherein such sugar linkages are resistant to endogenous  
nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e.,  
capable of resisting enzymatic degradation) but retain sequence specificity to be able to  
bind to target nucleotide sequences. Other examples of sense or antisense  
oligonucleotides include those oligonucleotides which are covalently linked to organic  
15 moieties, such as those described in WO 90/10448, and other moieties that increases  
affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine).  
Further still, intercalating agents, such as ellipticine, and alkylating agents or metal  
complexes may be attached to sense or antisense oligonucleotides to modify binding  
specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

20 Antisense or sense oligonucleotides may be introduced into a cell containing the  
target nucleic acid sequence by any gene transfer method, including, for example,  
CaPO<sub>4</sub>-mediated DNA transfection, electroporation, or by using gene transfer vectors  
such as Epstein-Barr virus. Antisense or sense oligonucleotides are preferably introduced  
into a cell containing the target nucleic acid sequence by insertion of the antisense or  
25 sense oligonucleotide into a suitable retroviral vector, then contacting the cell with the  
retrovirus vector containing the inserted sequence, either *in vivo* or *ex vivo*. Suitable  
retroviral vectors include, but are not limited to, those derived from the murine retrovirus  
M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors  
designated DCT5A, DCT5B and DCT5C (see PCT Application US 90/02656).

30 Sense or antisense oligonucleotides also may be introduced into a cell containing  
the target nucleotide sequence by formation of a conjugate with a ligand binding  
molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but  
are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands  
that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule  
35 does not substantially interfere with the ability of the ligand binding molecule to bind to  
its corresponding molecule or receptor, or block entry of the sense or antisense  
oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

5 In addition to the above, the following examples are provided to illustrate particular embodiments and not to limit the scope of the invention.

### **EXAMPLE 1**

#### **Isolating a Human Semaphorin Homologue Designated DCSema**

10 Using sequence alignment techniques and comparative sequence analysis of ESTs, a human homologue of AHV Sema semaphorin was identified as follows. The nucleotide sequence of viral A39R was used in a Unigene comparative sequence search. This search resulted in the identification of EST #151129 (accession # [H02902], deposited June 20, 1995. EST 151129 is a partial sequence having neither an initiating  
15 ATG nor a termination codon. EST 151129 has sequence homology to A39R and AHVsema.

EST 151129 was utilized to isolate and identify the native human DCSema as follows:

20 A tissue source of EST 151129 was identified using phage library screening methods and PCR primers based upon this EST sequence. Oligonucleotide PCR primers having the following nucleotide sequences were synthesized:

5'-TGCTGGAACCTGGTGAATGG-3' (SEQ ID NO:3)

25 5'-AGTGGAACAATGGCGTCTTC-3' (SEQ ID NO:4)

PCR isolation and amplification methodologies were carried out using a panel of human tissue cDNA phage libraries as templates for the PCR reactions.

30 Two of the phage libraries, human foreskin fibroblast and dermal fibroblast, were chosen for additional analysis. The libraries were plated according to established procedures. A radiolabeled probe was generated by incorporating <sup>32</sup>P-dCTP in the amplification of a PCR product using the EST 151129 as a template. The PCR reaction mixture included 10ng of EST151129 plasmid DNA, 50 pmoles of each PCR oligonucleotide primer identified below and specific for the 5' end of EST 151129, 1X  
35 Amplitaq buffer (Perkin-Elmer Cetus), 1.25 mM of dATP, dGTP, dTTP, .001mM dCTP (Pharmacia, ), 150μCi <sup>32</sup>P-dCTP, 0.5ul Amplitaq taq polymerase (Perkin-Elmer Cetus,) in a 100μl final reaction volume. The PCR reaction cycles included one cycle at 94°C for 5

min; twenty-five cycles at 94°C for 1 min, 72°C for 2 min and one cycle at 72°C for 5 min using a Robocycler Gradient 40 (Stratagene, La Jolla, CA).

The primers used were as follows:

5

5'-TCCGCCCAGGGCCACCTAAGGAGCGGA-3' (SEQ ID NO:7)

5'-TGTGCGGCTCAGTCTGGCCAAAGTCCA-3' (SEQ ID NO:8)

- 10 Approximately  $5 \times 10^5$  cpm/mL of purified probe was used to hybridize with the human dermal fibroblast library on nylon membrane filters in the same manner described in Example 5 for probing human foreskin fibroblast and human dermal fibroblast libraries.

A cDNA that overlapped with the 5' end of EST 151129 was isolated. Using sequence alignments it was determined that EST 151129 sequence included nucleotides  
 15 115-1536, encoding amino acids 39-512 of the full length peptide. The isolated cDNA provided nucleotides 1-114 and additional upstream non-coding nucleotides. These added nucleotides encode amino acids 1-38 of the full length human semaphorin, amino acid 1 being the initiating methionine.

- 20 In order to isolate cDNA overlapping the 3' end of EST 151129, another radiolabeled PCR probe was generated as described above but using PCR oligonucleotide primers specific for the 3' end of EST 151129:

5'-AGCCAGGTGCCCCTGGACCT-3' (SEQ ID NO:9)

- 25 5'-CTCGAGGCCAAGAATTCGGC-3' (SEQ ID NO:10)

Approximately  $5 \times 10^5$  cpm/mL of purified probe was used to hybridize with the human foreskin fibroblast library on nylon membrane filters following the hybridization and washing protocol described above. Three independent cDNA clones were isolated which  
 30 overlapped with the 3' end of EST151129 and extended the 3' end of EST 151129 from nucleotide 1537 to 2001. The isolated clones contained additional downstream non-coding nucleotides. Nucleotides 1537-2001 translate into amino acids 513-666 of the full length peptide plus a stop codon. The full cDNA sequence of the human DCSema homologue is provided in SEQ ID NO:1. The encoded amino acid sequence is shown in  
 35 SEQ ID NO:2.

**EXAMPLE 2****Northern Blot Analysis for Tissue Expressing the Human Homologue**

Radiolabeled probes derived from SEQ ID NO:1 were used in Northern blot analyses of different human tissues to identify the tissue distribution of the human homologue of AHV Sema, designated DCSema.

Human poly A+ multiple tissue northern blots were purchased from Clontech Laboratories, Palo Alto, CA (Cat. #s 7760-1, 7759-1, 7756-1, 757-1). The northern blot filters were prehybridized, probed, and washed according to manufacturer's instructions. The probe was an antisense riboprobe specific for EST 151129, an EST discovered using the viral AHV Sema sequence in sequence alignment analyses and comparative analysis methods. The template for the riboprobe was generated using PCR techniques and oligonucleotide primers that were designed to span nucleotides 613 to 978 of EST 151129(HO2902/H). The primers sequences were as follows:

15

5' Primer:

TCTACTACTT CTTCC (SEQ ID NO:5)

3' Primer

GGAATCCTAA TACGACTCAC TATAGGGAGG CGGGTTGGGA AGGC (SEQ ID NO:6)

The underlined portion of the downstream primer is a T7 site.

The riboprobe was generated using Ambion's MAXIscript SP6/T7 kit by combining 3 $\mu$ L of RNase free water, 2 $\mu$ L 10x transcription buffer, 1 $\mu$ L each of 10mM dATP/dCTP/dGTP, 5 $\mu$ L 5'/3' EST 151129 PCR Product, 5  $\mu$ L Amersham [ $\alpha^{32}$ P]UTP 10mCi/mL, and 2 $\mu$ L T7 RNA polymerase at room temperature. The combination was microfuged, spun briefly, and incubated at 37°C for 30 minutes. Then 1 $\mu$ L DNase was added to the mixture and allowed to react for 15 minutes at 37°C. The reaction product was passed through two column volumes of G-25 (Boehringer). One microliter (1 $\mu$ L) of the riboprobe was counted in a scintillation counter for 1 minute to determine cpm/ $\mu$ L.

After probing the northern blots, they were washed once for 30 minutes with 2x SSC, 0.05%SDS at 63°C and three times for 30 minutes with 0.1xSSC, 0.1% SDS and then exposed to x-ray film. The developed film indicated that the human DCSema homologue is found in placenta, brain, spinal cord, testes and spleen. Weak hybridization signals were observed in skeletal muscle, lymph node, ovary, and bone marrow.

**EXAMPLE 3**  
**Preparing a DC-Sema/Fc fusion protein**

This example describes preparing a DC-Sema/Fc DNA construct and subsequently expressing a DC-Sema/immunoglobulin Fc fusion protein referred to as DC-Sema/Fc. DNA encoding DC-Sema/Fc included a nucleotide sequence that encodes a murine IL-7 leader peptide, a FLAG™ octapeptide (described in U.S. Patent No. 5,011,912), an Fc region of an immunoglobulin mutated to minimize binding to Fc receptor (described by Baum et al. *Cir. Sh.* 44:30, 1994), a flexible linker sequence and DNA encoding amino acids 45-666 of SEQ ID NO:2. An expression vector containing the leader sequence, FLAG, mutated hu IgG Fc and flexible linker was prepared using conventional enzyme cutting and ligation techniques. The resulting vector was then restricted with SpeI and NotI. The DC-Sema was inserted 5' to 3' after the flexible linker in a two-way ligation described below.

To prepare the DC-Sema DNA, three primer pairs were designed and used to amplify three DNA fragments. Two of the fragments were amplified from a human foreskin fibroblast library phage clone containing the 3' portion of the DCSema cDNA and one fragment was EST DNA (Research Genetics Imageclone ID #151129). The three amplified DNA fragments were combined in a PCR SOEing reaction to generate a DNA fragment encoding the entire peptide of DC-Sema (Horton et al., *Biotechniques* 8:528, 1990). In the final fragment, the upstream oligonucleotide primer introduced a SpeI site upstream of amino acid 45 of the DC-sema peptide. A downstream oligonucleotide primer introduced a NotI site just downstream of the termination codon after amino acid 666.

The PCR fragment was then ligated into an expression vector (pDC409) containing the leader sequence, Flag® sequence, mutated human IgG Fc and a flexible linker region in a two-way ligation. The resulting DNA construct was transfected into the monkey kidney cell lines CV-1/EBNA.. After 7 days of culture in medium containing 0.5% low immunoglobulin bovine serum, a solution of 0.2% azide was added to the supernatant and the supernatant was filtered through a 0.22 µm filter. Then approximately 1 L of culture supernatant was passed through a BioCad Protein A HPLC protein purification system using a 4.6 x 100 mm Protein A column (POROS 20A from PerSeptive Biosystems) at 10 mL/min. The Protein A column binds the Fc Portion of the fusion protein in the supernatant, immobilizing the fusion protein and allowing other components of the supernatant to pass through the column. The column was washed with 30 mL of PBS solution and bound fusion protein was eluted from the HPLC column with citric acid adjusted to pH 3.0. Eluted purified fusion protein was neutralized as it eluted using 1M HEPES solution at pH 7.4.



**EXAMPLE 4**  
**Preparing DC-Sema/polyHIS Fusion Protein**

This example describes preparing a DC-Sema/polyHIS DNA construct and subsequently expressing a DC-Sema/poly histidine tagged protein referred to as DC-sema/polyHIS fusion protein. DNA encoding DC-sema/polyHIS comprises sequences encoding the DC-sema gene from amino acid 1 to amino acid 666 (no stop codon), followed by a factor Xa cleavage site, a FLAG<sup>®</sup> octapeptide, a string of six histidine residues and a stop codon. An expression vector containing the factor Xa cleavage site, flag, and polyHIS sequence was prepared using conventional enzyme cutting and fragment ligation techniques. The resulting vector was then restricted with SalI and SnaBI. The DC-Sema sequence was inserted 5' to 3' before the factor Xa cleavage site in a two-way ligation described below.

To prepare the DC-Sema DNA, three primer pairs were designed and used to amplify three DNA fragments. Two of the fragments were from phage DNA and one fragment was EST DNA (Research Genetics Imageclone ID #151129). The three amplified DNA fragments were combined in a PCR SOEing reaction to generate a DNA fragment encoding the entire peptide of DC-Sema. This final DNA included a SalI site upstream of amino acid 1 of the DC-Sema peptide and a SnaBI site downstream of amino acid 666.

The PCR product was then ligated into an expression vector pDC409 containing the factor Xa cleavage site, flag, and polyHIS sequence in a two-way ligation. The resultant DNA construct (DC-Sema/polyHIS) was transiently transfected into the monkey cell line COS-1 (ATCC CRL-1650). Following a 7 day culture in medium containing 0.5% low immunoglobulin bovine serum, cell supernatants were harvested and a solution of 0.2% sodium azide was added to the supernatants. The supernatants were filtered through a 0.22 µm filter, concentrated 10 fold with a prep scale concentrator (Millipore; Bedford, MA) and purified on a BioCad HPLC protein purification equipped with a Nickel NTA Superflow self pack resin column (Qiagen, Santa Clarita, CA). After the supernatant passed through the column, the column was washed with Buffer A (20mM NaPO<sub>4</sub>, pH7.4; 300mM NaCl; 50mM Imidazole). Bound protein was then eluted from the column using a gradient elution techniques. Fractions containing protein were collected and analyzed on a 4-20% SDS-PAGE reducing gel. Peaks containing the fusion protein were pooled, concentrated 2 fold, and then dialyzed in PBS. The resulting DC-Sema/polyHis fusion protein was then filtered through a 0.22µm sterile filter and recovered.

**EXAMPLE 5****Screening Cell Lines for Binding to DC-Sema**

The DC-Sema/Fc fusion protein prepared as described in Example 3 was used to screen cell lines for binding using quantitative binding studies according to standard flow cytometry methodologies. For each cell line screened, the procedure involved incubating approximately 100,000 of the cells blocked with 2% FCS (fetal calf serum), 5% normal goat serum and 5% rabbit serum in PBS for 1 hour. Then the blocked cells were incubated with 5 µg/mL of DC-Sema/Fc fusion protein in 2% FCS, 5% goat serum and 5% rabbit serum in PBS. Following the incubation the sample was washed 2 times with FACS buffer (2% FCS in PBS) and then treated with mouse anti human Fc/biotin (purchased from Jackson Research) and SAPE (streptavidin-phycoerythrin purchased from Molecular Probes). This treatment causes the antihuman Fc/biotin to bind to any bound DC-Sema/Fc and the SAPE to bind to the anti human Fc/biotin resulting in a fluorescent identifying label on DC-Sema/Fc which is bound to cells. The cells were analyzed for any bound protein using fluorescent detection flow cytometry. Table I details the results of the flow cytometry studies. + indicates that binding was detected between the cell surface and DC-Sema. - indicates that no binding was detected between the cell surface and A39R.

**TABLE I**

<u>Cell Line</u>	<u>DC-Sema Binding Result</u>
CB23 (Human Cord Blood B Cell Line)	+
MP-1 (Human B Cell Lymphoma)	+
PB B (Human Peripheral Blood B Cells)	+
U937 (Human Monocyte-Type Cell)	+
W126 (Human Lung Epithelium)	+
RAJI (Burkitt's Lymphoma)	+
Primary Human Monocytes	+
THP-1 (Human Promonocytes)	+

**Example 6****Monoclonal Antibodies to DC-Sema**

This example illustrates a method for preparing antibodies to DC-Sema. Purified DC-Sema/Fc is prepared as described in Example 3 above. The purified protein is used to generate antibodies against DC-Sema as described in U.S. Patent 4,411,993. Briefly, mice are immunized at 0, 2 and 6 weeks with 10 µg with DC-Sema/Fc. The primary immunization is prepared with TITERMAX adjuvant, from Vaxcell, Inc., and subsequent immunizations are prepared with incomplete Freund's adjuvant (IFA). At 11 weeks, the

mice are IV boosted with 3-4  $\mu$ g DCSema/Fc in PBS. Three days after the IV boost, splenocytes are harvested and fused with an Ag8.653 myeloma fusion partner using 50% aqueous PEG 1500 solution. Hybridoma supernatants are screened for DC-Sema antibodies by dot blot assay against DCSema/FC and an irrelevant Fc protein.

5

### Example 7

#### Cytokine Induction from Freshly Isolated Human Monocytes

Freshly isolated human monocytes were purified by first diluting 1:1 peripheral blood from healthy donors in low endotoxin PBS at pH 7.4 and room temperature. Then  
10 35 mLs of the diluted blood was layered over 15 mLs of IsolympH (Gallard and Schlesinger Industries, Inc; Carle Place, NY) and centrifuged at 2200 rpm for 25 minutes at room temperature. The plasma layers was reserved. The PBMC layer was harvested and washed three times to remove the IsolympH. The washed PBMC's were resuspended in X-Vivo 15 serum free media (BioWhittaker, Walkersville, MD) and added to T175  
15 flasks. The flasks had been previously coated with 2% Gelatin (Sigma, St. Louis, MO) and pre-treated for 30 minutes with the reserved plasma layer. The PBMC's were allowed to adhere for 90 minutes at 37°C, 5% CO<sub>2</sub> and then rinsed three times gently with 10 mL washes of low endotoxin PBS. Adhered monocytes were harvested by incubating the cells in Enzyme Free Dissociation Buffer (Gibco, BRL) and washing the cells  
20 multiple times in PBS. Monocytes were centrifuged at 2500 rpm for 5 minutes, counted, and set up in 24 well dishes at 5 x 10<sup>5</sup> cells/well in 1 mL. The cultures were 95% pure.

Purified monocytes were cultured for 7-9 days in the presence of 20 ng/mL GM-CSF and 100 ng/mL IL-4 in order to allow cells to differentiate to a more dendritic cell-like phenotype. On day 7-9, cultures were treated with 1  $\mu$ g/mL DCSema/Fc fusion  
25 protein (see Example 3) or a control Fc protein, and the next day cells and supernatants were harvested for analyses.

The monocyte supernatants were examined for the presence of proinflammatory cytokines. In all donors tested, IL-6 and IL-8 was induced by DCSema protein. Heat inactivated DCSema and control proteins did not induce IL-6 or IL-8. Additionally,  
30 cytokine production was blocked by the inclusion of a mAb directed against DCSema.

The results of this experiment demonstrate that DCSema, or homologues of this protein, by interact with its receptor, can induce cytokine production by freshly isolated monocytes.

35

**Example 8****Monocyte Aggregation Studies**

5 In order to examine human monocyte response to the interaction of a DCSema to its receptor on monocytes, monocytes were purified as described in Example 7 and DC-Sema/Fc fusion protein was prepared as described in Example 3. After incubating the DCSema/Fc fusion protein and purified, cultured monocytes for 20 hours, monocyte aggregation was observed.

10 This work confirms that the receptor for the DCSema of the present invention is expressed on monocytes and that the interaction between DCSema and its receptor results in monocyte aggregation. Similar to B cells, monocyte aggregation is indicative of their activation.

What is claimed is:

1. A semaphorin polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO:2, the semaphorin polypeptide capable of binding at least one semaphorin receptor.
2. An isolated semaphorin polypeptide encoded by DNA sequences selected from the group consisting of:
  - (a) nucleotides  $x_1$  - 2001 of SEQ ID NO:1, wherein  $x_1$  is nucleotide 1 or 135; and
  - (b) DNA sequences that hybridize under moderately stringent conditions to the DNA of (a); and which DNA sequences encode a polypeptide that binds semaphorin receptors.
3. A semaphorin polypeptide comprising an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of:
  - (a) amino acids  $x_1$  to 666 of SEQ ID NO: 2, wherein  $x_1$  is amino acid 1 or 45.a fragment of the sequence of (a), wherein the fragment is capable of binding a semaphorin receptor.
4. A semaphorin polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) amino acids  $x_1$  to 666 of SEQ ID NO: 2, wherein  $x_1$  is amino acid 1 or 45, and
  - (b) a fragment of the sequence of (a), wherein the fragment is capable of binding a semaphorin receptor.
5. An isolated DNA encoding a semaphorin polypeptide, the DNA selected from the group consisting of:
  - (a) DNA capable of hybridizing under moderately stringent conditions to a nucleotide sequence consisting essentially of SEQ ID NO:1; and
  - (b) DNA capable of hybridizing under moderately stringent conditions to DNA complementary to the sequence of (a).
6. An isolated DNA encoding a semaphorin polypeptide, the DNA selected from the group consisting of:

- (a) nucleotides  $x_1$  - 2001 of SEQ ID NO:1, wherein  $x_1$  is nucleotide 1 or 135;  
and
- (b) DNA sequences that hybridize under moderately stringent conditions to the cDNA of (a); and which DNA sequences encode a polypeptide that binds semaphorin receptor; and
- (c) DNA sequences that, due to the degeneracy of the genetic code, encode semaphorin polypeptides having the amino acid sequence of the polypeptides encoded by the DNA sequences of (a) or (b).
7. An isolated DNA encoding an semaphorin polypeptide wherein the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO:2.
8. The isolated DNA of claim 7 wherein the semaphorin polypeptide comprises the amino acid sequence of SEQ ID NO:2.
9. An isolated DNA encoding a semaphorin polypeptide, wherein the polypeptide comprises an amino acid sequence that is at least 80% identical to a sequence selected from the group consisting of:
- (a) amino acids  $x_1$  - 666 of SEQ ID NO:2, wherein  $x_1$  is amino acid 1 or 45;  
and
- (b) a fragment of the sequence of (a), wherein the polypeptide binds a semaphorin receptor.
10. A DNA of claim 9 wherein the semaphorin polypeptide comprises an amino acid sequence selected from the group consisting of:
- (a) amino acids  $x_1$  - 666 of SEQ ID NO:2, wherein  $x_1$  is amino acid 1 or 45;  
and
- (b) a fragment of (a).
11. A DNA that is at least 80% identical to DNA that encodes an amino acid sequence selected from the group consisting:
- (a) amino acids  $x_1$  - 666 of SEQ ID NO:2, wherein  $x_1$  is amino acid 1 or 45;  
and
- (b) a fragment of (a).

12. A fusion protein comprising amino acids  $x_1$  - 666 where  $x_1$  is amino acid 1 or 45 of SEQ ID NO:2
13. A recombinant expression vector comprising DNA of claim 6.
14. A process for preparing a semaphorin polypeptide, the process comprising culturing a host cell transformed with an expression vector of claim 14 under conditions that promote expression of the polypeptide, and recovering the polypeptide.
15. A composition comprising a suitable diluent carrier and a polypeptide of claim 3.
16. An antibody that is immunoreactive with a polypeptide of claim 2.
17. A process for treating an inflammatory disease in a mammal afflicted with the disease, the process comprising administering an amount of semaphorin polypeptide.
18. A method of separating cells having semaphorin polypeptide on the surface thereof from a mixture of cells in suspension, comprising contacting the cells in the mixture with a contacting surface having a semaphorin, and separating the contacting surface and the suspension.
19. An isolated DNA selected from the group consisting of:
  - (a) DNA of SEQ ID NO:5;
  - (b) DNA sequences that hybridize under moderately stringent conditions to the cDNA of (a); and which DNA sequences encode a polypeptide that binds semaphorin receptor;
  - (c) DNA sequences that, due to the degeneracy of the genetic code, encode semaphorin polypeptides having the amino acid sequence of the polypeptides encoded by the DNA sequences of (a) or (b).
20. An isolated DNA encoding a soluble semaphorin polypeptide, wherein the soluble polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of:
  - (a) amino acids  $x_1$  to 666 of SEQ ID NO:6, wherein  $x_1$  is amino acid 1 or 45;
  - (b) a fragment of the sequence of (a), wherein the soluble polypeptide binds a semaphorin receptor.

21. A DNA of claim 29 wherein the soluble polypeptide comprises an amino acid sequence selected from the group consisting of:
- (a) amino acids  $x_1$  to 666 of SEQ ID NO:6, wherein  $x_1$  is amino acid 1 or 45; and
  - (b) a fragment of (a).
22. A recombinant expression vector comprising DNA of claim 21.
23. A process for preparing a semaphorin polypeptide, the process comprising culturing a host cell transformed with an expression vector of claim 31 under conditions that promote expression of the polypeptide, and recovering the polypeptide.



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Immunex Corporation and Spriggs, Melanie K.
- (ii) TITLE OF INVENTION: NOVEL SEMAPHORIN POLYPEPTIDES
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
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  - (C) CITY: Seattle
  - (D) STATE: WA
  - (E) COUNTRY: US
  - (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) INTER'L APPLICATION NUMBER: --to be assigned--
  - (B) FILING DATE: 05 MAY 1999
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/085,497
  - (B) FILING DATE: May 14, 1998
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Henry, Janis C
  - (B) REGISTRATION NUMBER: 34,347
  - (C) REFERENCE/DOCKET NUMBER: 2634-WO
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (206)470-4189
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2001 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (ix) FEATURE:
  - (A) NAME/KEY: CDS

(B) LOCATION: 1..2001

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG ACG CCT CCT CCG CCC GGA CGT GCC GCC CCC AGC GCA CCG CGC GCC	48
Met Thr Pro Pro Pro Gly Arg Ala Ala Pro Ser Ala Pro Arg Ala	
1 5 10 15	
CGC GTC CCT GGC CCG CCG GCT CGG TTG GGG CTT CCG CTG CGG CTG CGG	96
Arg Val Pro Gly Pro Pro Ala Arg Leu Gly Leu Pro Leu Arg Leu Arg	
20 25 30	
CTG CTG CTG CTG CTT TGG GCG GCC GCC GCC TCC GCC CAG GGC CAC CTA	144
Leu Leu Leu Leu Leu Trp Ala Ala Ala Ser Ala Gln Gly His Leu	
35 40 45	
AGG AGC GGA CCC CGC ATC TTC GCC GTC TGG AAA GGC CAT GTA GGG CAG	192
Arg Ser Gly Pro Arg Ile Phe Ala Val Trp Lys Gly His Val Gly Gln	
50 55 60	
GAC CGG GTG GAC TTT GGC CAG ACT GAG CCG CAC ACG GTG CTT TTC CAC	240
Asp Arg Val Asp Phe Gly Gln Thr Glu Pro His Thr Val Leu Phe His	
65 70 75 80	
GAG CCA GGC AGC TCC TCT GTG TGG GTG GGA GGA CGT GGC AAG GTC TAC	288
Glu Pro Gly Ser Ser Ser Val Trp Val Gly Gly Arg Gly Lys Val Tyr	
85 90 95	
CTC TTT GAC TTC CCC GAG GGC AAG AAC GCA TCT GTG CGC ACG GTG AAT	336
Leu Phe Asp Phe Pro Glu Gly Lys Asn Ala Ser Val Arg Thr Val Asn	
100 105 110	
ATC GGC TCC ACA AAG GGG TCC TGT CTG GAT AAG CGG GAC TGC GAG AAC	384
Ile Gly Ser Thr Lys Gly Ser Cys Leu Asp Lys Arg Asp Cys Glu Asn	
115 120 125	
TAC ATC ACT CTC CTG GAG AGG CGG AGT GAG GGG CTG CTG GCC TGT GGC	432
Tyr Ile Thr Leu Leu Glu Arg Arg Ser Glu Gly Leu Leu Ala Cys Gly	
130 135 140	
ACC AAC GCC CGG CAC CCC AGC TGC TGG AAC CTG GTG AAT GGC ACT GTG	480
Thr Asn Ala Arg His Pro Ser Cys Trp Asn Leu Val Asn Gly Thr Val	
145 150 155 160	
GTG CCA CTT GGC GAG ATG AGA GGC TAC GCC CCC TTC AGC CCG GAC GAG	528
Val Pro Leu Gly Glu Met Arg Gly Tyr Ala Pro Phe Ser Pro Asp Glu	
165 170 175	
AAC TCC CTG GTT CTG TTT GAA GGG GAC GAG GTG TAT TCC ACC ATC CGG	576
Asn Ser Leu Val Leu Phe Glu Gly Asp Glu Val Tyr Ser Thr Ile Arg	
180 185 190	
AAG CAG GAA TAC AAT GGG AAG ATC CCT CGG TTC CGC CGC ATC CGG GGC	624
Lys Gln Glu Tyr Asn Gly Lys Ile Pro Arg Phe Arg Arg Ile Arg Gly	
195 200 205	
GAG AGT GAG CTG TAC ACC AGT GAT ACT GTC ATG CAG AAC CCA CAG TTC	672
Glu Ser Glu Leu Tyr Thr Ser Asp Thr Val Met Gln Asn Pro Gln Phe	
210 215 220	
ATC AAA GCC ACC ATC GTG CAC CAA GAC CAG GCT TAC GAT GAC AAG ATC	720
Ile Lys Ala Thr Ile Val His Gln Asp Gln Ala Tyr Asp Asp Lys Ile	
225 230 235 240	

TAC	TAC	TTC	TTC	CGA	GAG	GAC	AAT	CCT	GAC	AAG	AAT	CCT	GAG	GCT	CCT	768
Tyr	Tyr	Phe	Phe	Arg	Glu	Asp	Asn	Pro	Asp	Lys	Asn	Pro	Glu	Ala	Pro	
				245					250					255		
CTC	AAT	GTG	TCC	CGT	GTG	GCC	CAG	TTG	TGC	AGG	GGG	GAC	CAG	GGT	GGG	816
Leu	Asn	Val	Ser	Arg	Val	Ala	Gln	Leu	Cys	Arg	Gly	Asp	Gln	Gly	Gly	
			260					265					270			
GAA	AGT	TCA	CTG	TCA	GTC	TCC	AAG	TGG	AAC	ACT	TTT	CTG	AAA	GCC	ATG	864
Glu	Ser	Ser	Leu	Ser	Val	Ser	Lys	Trp	Asn	Thr	Phe	Leu	Lys	Ala	Met	
		275					280					285				
CTG	GTA	TGC	AGT	GAT	GCT	GCC	ACC	AAC	AAG	AAC	TTC	AAC	AGG	CTG	CAA	912
Leu	Val	Cys	Ser	Asp	Ala	Ala	Thr	Asn	Lys	Asn	Phe	Asn	Arg	Leu	Gln	
	290					295					300					
GAC	GTC	TTC	CTG	CTC	CCT	GAC	CCC	AGC	GGC	CAG	TGG	AGG	GAC	ACC	AGG	960
Asp	Val	Phe	Leu	Leu	Pro	Asp	Pro	Ser	Gly	Gln	Trp	Arg	Asp	Thr	Arg	
305					310					315					320	
GTC	TAT	GGT	GTT	TTC	TCC	AAC	CCC	TGG	AAC	TAC	TCA	GCC	GTC	TGT	GTG	1008
Val	Tyr	Gly	Val	Phe	Ser	Asn	Pro	Trp	Asn	Tyr	Ser	Ala	Val	Cys	Val	
				325				330						335		
TAT	TCC	CTC	GGT	GAC	ATT	GAC	AAG	GTC	TTC	CGT	ACC	TCC	TCA	CTC	AAG	1056
Tyr	Ser	Leu	Gly	Asp	Ile	Asp	Lys	Val	Phe	Arg	Thr	Ser	Ser	Leu	Lys	
			340					345					350			
GGC	TAC	CAC	TCA	AGC	CTT	CCC	AAC	CCG	CGG	CCT	GGC	AAG	TGC	CTC	CCA	1104
Gly	Tyr	His	Ser	Ser	Leu	Pro	Asn	Pro	Arg	Pro	Gly	Lys	Cys	Leu	Pro	
		355					360					365				
GAC	CAG	CAG	CCG	ATA	CCC	ACA	GAG	ACC	TTC	CAG	GTG	GCT	GAC	CGT	CAC	1152
Asp	Gln	Gln	Pro	Ile	Pro	Thr	Glu	Thr	Phe	Gln	Val	Ala	Asp	Arg	His	
	370					375					380					
CCA	GAG	GTG	GCG	CAG	AGG	GTG	GAG	CCC	ATG	GGG	CCT	CTG	AAG	ACG	CCA	1200
Pro	Glu	Val	Ala	Gln	Arg	Val	Glu	Pro	Met	Gly	Pro	Leu	Lys	Thr	Pro	
385					390					395					400	
TTG	TTC	CAC	TCT	AAA	TAC	CAC	TAC	CAG	AAA	GTG	GCC	GTT	CAC	CGC	ATG	1248
Leu	Phe	His	Ser	Lys	Tyr	His	Tyr	Gln	Lys	Val	Ala	Val	His	Arg	Met	
				405					410					415		
CAA	GCC	AGC	CAC	GGG	GAG	ACC	TTT	CAT	GTG	CTT	TAC	CTA	ACT	ACA	GAC	1296
Gln	Ala	Ser	His	Gly	Glu	Thr	Phe	His	Val	Leu	Tyr	Leu	Thr	Thr	Asp	
			420					425					430			
AGG	GGC	ACT	ATC	CAC	AAG	GTG	GTG	GAA	CCG	GGG	GAG	CAG	GAG	CAC	AGC	1344
Arg	Gly	Thr	Ile	His	Lys	Val	Val	Glu	Pro	Gly	Glu	Gln	Glu	His	Ser	
		435					440					445				
TTC	GCC	TTC	AAC	ATC	ATG	GAG	ATC	CAG	CCC	TTC	CGC	CGC	GCG	GCT	GCC	1392
Phe	Ala	Phe	Asn	Ile	Met	Glu	Ile	Gln	Pro	Phe	Arg	Arg	Ala	Ala	Ala	
	450					455					460					
ATC	CAG	ACC	ATG	TCG	CTG	GAT	GCT	GAG	CGG	AGG	AAG	CTG	TAT	GTG	AGC	1440
Ile	Gln	Thr	Met	Ser	Leu	Asp	Ala	Glu	Arg	Arg	Lys	Leu	Tyr	Val	Ser	
465					470				475						480	
TCC	CAG	TGG	GAG	GTG	AGC	CAG	GTG	CCC	CTG	GAC	CTG	TGT	GAG	GTC	TAT	1488
Ser	Gln	Trp	Glu	Val	Ser	Gln	Val	Pro	Leu	Asp	Leu	Cys	Glu	Val	Tyr	
				485					490					495		

GGC GGG GGC TGC CAC GGT TGC CTC ATG TCC CGA GAC CCC TAC TGC GGC	1536
Gly Gly Gly Cys His Gly Cys Leu Met Ser Arg Asp Pro Tyr Cys Gly	
500 505 510	
TGG GAC CAG GGC CGC TGC ATC TCC ATC TAC AGC TCC GAA CGG TCA GTG	1584
Trp Asp Gln Gly Arg Cys Ile Ser Ile Tyr Ser Ser Glu Arg Ser Val	
515 520 525	
CTG CAA TCC ATT AAT CCA GCC GAG CCA CAC AAG GAG TGT CCC AAC CCC	1632
Leu Gln Ser Ile Asn Pro Ala Glu Pro His Lys Glu Cys Pro Asn Pro	
530 535 540	
AAA CCA GAC AAG GCC CCA CTG CAG AAG GTT TCC CTG GCC CCA AAC TCT	1680
Lys Pro Asp Lys Ala Pro Leu Gln Lys Val Ser Leu Ala Pro Asn Ser	
545 550 555 560	
CGC TAC TAC CTG AGC TGC CCC ATG GAA TCC CGC CAC GCC ACC TAC TCA	1728
Arg Tyr Tyr Leu Ser Cys Pro Met Glu Ser Arg His Ala Thr Tyr Ser	
565 570 575	
TGG CGC CAC AAG GAG AAC GTG GAG CAG AGC TGC GAA CCT GGT CAC CAG	1776
Trp Arg His Lys Glu Asn Val Glu Gln Ser Cys Glu Pro Gly His Gln	
580 585 590	
AGC CCC AAC TGC ATC CTG TTC ATC GAG AAC CTC ACG GCG CAG CAG TAC	1824
Ser Pro Asn Cys Ile Leu Phe Ile Glu Asn Leu Thr Ala Gln Gln Tyr	
595 600 605	
GGC CAC TAC TTC TGC GAG GCC CAG GAG GGC TCC TAC TTC CGC GAG GCT	1872
Gly His Tyr Phe Cys Glu Ala Gln Glu Gly Ser Tyr Phe Arg Glu Ala	
610 615 620	
CAG CAC TGG CAG CTG CTG CCC GAG GAC GGC ATC ATG GCC GAG CAC CTG	1920
Gln His Trp Gln Leu Leu Pro Glu Asp Gly Ile Met Ala Glu His Leu	
625 630 635 640	
CTG GGT CAT GCC TGT GCC CTG GCT GCC TCC CTC TGG CTG GGC GTG CTG	1968
Leu Gly His Ala Cys Ala Leu Ala Ala Ser Leu Trp Leu Gly Val Leu	
645 650 655	
CCC ACA CTC ACT CTT GGC TTG CTG GTC CAC TAG	2001
Pro Thr Leu Thr Leu Gly Leu Leu Val His *	
660 665	

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 666 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Pro Pro Pro Pro Gly Arg Ala Ala Pro Ser Ala Pro Arg Ala	
1 5 10 15	
Arg Val Pro Gly Pro Pro Ala Arg Leu Gly Leu Pro Leu Arg Leu Arg	
20 25 30	
Leu Leu Leu Leu Leu Trp Ala Ala Ala Ser Ala Gln Gly His Leu	

35					40					45					
Arg	Ser	Gly	Pro	Arg	Ile	Phe	Ala	Val	Trp	Lys	Gly	His	Val	Gly	Gln
50						55					60				
Asp	Arg	Val	Asp	Phe	Gly	Gln	Thr	Glu	Pro	His	Thr	Val	Leu	Phe	His
65					70					75					80
Glu	Pro	Gly	Ser	Ser	Ser	Val	Trp	Val	Gly	Gly	Arg	Gly	Lys	Val	Tyr
				85					90					95	
Leu	Phe	Asp	Phe	Pro	Glu	Gly	Lys	Asn	Ala	Ser	Val	Arg	Thr	Val	Asn
			100					105					110		
Ile	Gly	Ser	Thr	Lys	Gly	Ser	Cys	Leu	Asp	Lys	Arg	Asp	Cys	Glu	Asn
		115					120					125			
Tyr	Ile	Thr	Leu	Leu	Glu	Arg	Ser	Glu	Gly	Leu	Leu	Ala	Cys	Gly	
	130					135					140				
Thr	Asn	Ala	Arg	His	Pro	Ser	Cys	Trp	Asn	Leu	Val	Asn	Gly	Thr	Val
145					150					155					160
Val	Pro	Leu	Gly	Glu	Met	Arg	Gly	Tyr	Ala	Pro	Phe	Ser	Pro	Asp	Glu
				165					170					175	
Asn	Ser	Leu	Val	Leu	Phe	Glu	Gly	Asp	Glu	Val	Tyr	Ser	Thr	Ile	Arg
			180					185					190		
Lys	Gln	Glu	Tyr	Asn	Gly	Lys	Ile	Pro	Arg	Phe	Arg	Arg	Ile	Arg	Gly
		195					200					205			
Glu	Ser	Glu	Leu	Tyr	Thr	Ser	Asp	Thr	Val	Met	Gln	Asn	Pro	Gln	Phe
		210				215					220				
Ile	Lys	Ala	Thr	Ile	Val	His	Gln	Asp	Gln	Ala	Tyr	Asp	Asp	Lys	Ile
225					230					235					240
Tyr	Tyr	Phe	Phe	Arg	Glu	Asp	Asn	Pro	Asp	Lys	Asn	Pro	Glu	Ala	Pro
				245					250					255	
Leu	Asn	Val	Ser	Arg	Val	Ala	Gln	Leu	Cys	Arg	Gly	Asp	Gln	Gly	Gly
			260					265					270		
Glu	Ser	Ser	Leu	Ser	Val	Ser	Lys	Trp	Asn	Thr	Phe	Leu	Lys	Ala	Met
		275					280					285			
Leu	Val	Cys	Ser	Asp	Ala	Ala	Thr	Asn	Lys	Asn	Phe	Asn	Arg	Leu	Gln
		290				295					300				
Asp	Val	Phe	Leu	Leu	Pro	Asp	Pro	Ser	Gly	Gln	Trp	Arg	Asp	Thr	Arg
305					310					315					320
Val	Tyr	Gly	Val	Phe	Ser	Asn	Pro	Trp	Asn	Tyr	Ser	Ala	Val	Cys	Val
				325					330					335	
Tyr	Ser	Leu	Gly	Asp	Ile	Asp	Lys	Val	Phe	Arg	Thr	Ser	Ser	Leu	Lys
			340					345					350		
Gly	Tyr	His	Ser	Ser	Leu	Pro	Asn	Pro	Arg	Pro	Gly	Lys	Cys	Leu	Pro
		355					360					365			
Asp	Gln	Gln	Pro	Ile	Pro	Thr	Glu	Thr	Phe	Gln	Val	Ala	Asp	Arg	His
		370					375					380			

```

Pro Glu Val Ala Gln Arg Val Glu Pro Met Gly Pro Leu Lys Thr Pro
385                               390                               395                               400
Leu Phe His Ser Lys Tyr His Tyr Gln Lys Val Ala Val His Arg Met
                               405                               410                               415
Gln Ala Ser His Gly Glu Thr Phe His Val Leu Tyr Leu Thr Thr Asp
                               420                               425                               430
Arg Gly Thr Ile His Lys Val Val Glu Pro Gly Glu Gln Glu His Ser
                               435                               440                               445
Phe Ala Phe Asn Ile Met Glu Ile Gln Pro Phe Arg Arg Ala Ala Ala
                               450                               455                               460
Ile Gln Thr Met Ser Leu Asp Ala Glu Arg Arg Lys Leu Tyr Val Ser
465                               470                               475                               480
Ser Gln Trp Glu Val Ser Gln Val Pro Leu Asp Leu Cys Glu Val Tyr
                               485                               490                               495
Gly Gly Gly Cys His Gly Cys Leu Met Ser Arg Asp Pro Tyr Cys Gly
                               500                               505                               510
Trp Asp Gln Gly Arg Cys Ile Ser Ile Tyr Ser Ser Glu Arg Ser Val
                               515                               520                               525
Leu Gln Ser Ile Asn Pro Ala Glu Pro His Lys Glu Cys Pro Asn Pro
                               530                               535                               540
Lys Pro Asp Lys Ala Pro Leu Gln Lys Val Ser Leu Ala Pro Asn Ser
545                               550                               555                               560
Arg Tyr Tyr Leu Ser Cys Pro Met Glu Ser Arg His Ala Thr Tyr Ser
                               565                               570                               575
Trp Arg His Lys Glu Asn Val Glu Gln Ser Cys Glu Pro Gly His Gln
                               580                               585                               590
Ser Pro Asn Cys Ile Leu Phe Ile Glu Asn Leu Thr Ala Gln Gln Tyr
                               595                               600                               605
Gly His Tyr Phe Cys Glu Ala Gln Glu Gly Ser Tyr Phe Arg Glu Ala
610                               615                               620
Gln His Trp Gln Leu Leu Pro Glu Asp Gly Ile Met Ala Glu His Leu
625                               630                               635                               640
Leu Gly His Ala Cys Ala Leu Ala Ala Ser Leu Trp Leu Gly Val Leu
                               645                               650                               655
Pro Thr Leu Thr Leu Gly Leu Leu Val His *
                               660                               665

```

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: primer
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCTGGAACC TGGTGAATGG

20

- (2) INFORMATION FOR SEQ ID NO:4:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 10 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: primer
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGTGGAACAA TGGCGTCTTC

20

- (2) INFORMATION FOR SEQ ID NO:5:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 15 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: cDNA
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTACTACTT CTTCC

15

- (2) INFORMATION FOR SEQ ID NO:6:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 44 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: primer

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATCCTAA TACGACTCAC TATAGGGAGG CGGTTGGGA AGGC

44

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGCCCAGG GCCACCTAAG GAGCGGA

27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGTGCGGCTC AGTCTGGCCA AAGTCCA

27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) HYPOTHETICAL: NO



(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGCCAGGTGC CCCTGGACCT

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTCGAGGCCA AGAATTCGGC

20

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